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Identification of Membrane Antigen C33 Recognized by Monoclonal Antibodies Inhibitory to Human T-Cell Leukemia Virus Type 1 (HTLV-1)-Induced Syncytium Formation: Altered Glycosylation of C33 Antigen in HTLV-1-Positive T Cells

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We isolated four monoclonal antibodies (MAbs), M38, M101, M104, and C33, which were capable of inhibiting syncytium formation induced in a human T-cell line, MOLT-4-#8, by coculture with human T-cell leukemia virus type 1 (HTLV-1)-positive human T-cell lines. The MAbs had, however, no inhibitory activity on syncytium formation induced in a human osteosarcoma line, HOS, by HTLV-1-positive T-cell lines. They also did not inhibit syncytium formation induced in MOLT-4-#8 by human immunodeficiency virus type 1-positive MOLT-4. All MAbs reacted with various human cell lines of lymphoid and nonlymphoid origins, including HTLV-1-positive T-cell lines. Furthermore, they all reacted with a murine A9 clone containing human chromosome 11 fragment q23-pter. Two MAbs, M104 and C33, immunoprecipitated a membrane antigen with the same molecular size. The antigen (henceforth called C33 antigen) was about 40 to 55 kDa in HTLV-1-negative Jurkat, CEM, MOLT-4, and normal peripheral blood CD4-positive human T cells and about 40 to 75 kDa in HTLV-1-positive C91/PL, TCL-Kan, MT-2, and in fresh HTLV-1-transformed CD4-positive human T-cell lines. Pulse-chase experiments revealed that C33 antigen was synthesized as a 35-kDa precursor that was then processed to 41 to 50 kDa in MOLT-4 and to 44 to 70 kDa in C91/PL. In the presence of tunicamycin, a 28-kDa protein was synthesized. The conversion from 35 kDa to 41 to 50 kDa in MOLT-4 and to 44 to 70 kDa in C91/PL was inhibited by monensin. Treatment with *N*-glycanase alone, but not with sialidase and *O*-glycanase in combination, completely removed the sugar moiety of C33 antigen from both HTLV-1-negative Jurkat and HTLV-1-positive C91/PL. Therefore, C33 antigen has only N-linked carbohydrates, the modification of which appears to be substantially altered in the presence of the HTLV-1 genome.

Human T-cell leukemia virus type 1 (HTLV-1) is a type C retrovirus etiologically associated with adult T-cell leukemia (12, 14, 29, 40). Like many other retroviruses, HTLV-1 has the potential to induce syncytium formation, which can be demonstrated by using selected target cells (9, 13, 26, 27, 38). The viral envelope glycoproteins expressed on HTLV-1-infected cells are primarily responsible for this phenomenon (19). In the case of human immunodeficiency virus type 1 (HIV-1), another important human retrovirus causing AIDS, the viral envelope protein is first synthesized as a precursor polyprotein, gp160, from which gp120 and gp41 are produced through proteolytic processing (31, 37). gp120 is the viral cell attachment protein capable of binding to the receptor CD4 on helper T cells (3, 20, 24), whereas gp41 has an N-terminal hydrophobic region and functions as a fusogenic protein promoting fusion of viral envelope and cell membrane (6). Syncytium formation induced by HIV-1-infected cells is inhibited by monoclonal antibodies (MAbs) to CD4, the HIV-1 receptor (3, 20), and also by those to LFA-1, an intercellular adhesion molecule, or CD18 (the common subunit of LFA-1 and other leukocyte cell adhesion molecules) (10, 36a). In the case of HTLV-1, the precursor envelope protein is gp62, from which gp46 and gp21 are produced (8, 19, 22, 32, 35). gp46, like gp120 of HIV-1, is considered to be the viral cell attachment protein, but the identity of the HTLV-1 receptor has not been determined. gp21, like gp41 of HIV-1, probably functions in membrane fusion through its N-terminal hydrophobic region (for reviews, see references 27 and 38). To identify cell surface molecules involved in HTLV-1-induced syncytium formation, we have isolated four MAbs which are capable of inhibiting syncytium formation induced by HTLV-1. By using these MAbs, we have identified a membrane antigen (C33 antigen) which appears to be differentially modified in HTLV-1-positive human T-cell lines.

MATERIALS AND METHODS

Cells. MOLT-4-#8 (17), TOM-1 (18), and MOLT-4 persistently infected with HIV-1 (IIIB strain) (17) were provided by N. Yamamoto (Yamaguchi University Medical School, Yamaguchi, Japan). HOS (23) and HTLV-1-positive C91/PL (29a) were provided by H. Hoshino (Gunma University Medical School, Gunma, Japan), as were the HTLV-1-positive and -negative feline cell lines 8C and C77 (13). Mouse myeloma SP2 (7) was provided by K. Tada (Tohoku University School of Medicine, Sendai, Japan). Mouse A9 cell lines each containing a single human chromosome (an X/autosome translocation) (21) were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). All other cell lines have been in our possession for a long time. Fresh CD4-positive human T cells were isolated as follows. Peripheral blood mononuclear cells were isolated from heparinized venous blood samples by the standard isopycnic centrifugation method using Ficoll-Paque (Pharmacia). Fresh CD4-positive human T cells were purified by staining with an R-phycoerythrin-conjugated anti-T4 mouse MAb

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(DAKO Japan, Tokyo, Japan) and sorting with a FACStar (Becton Dickinson, Mountain View, Calif.). Transformation of human T cells with HTLV-1 was carried out by coculture of peripheral blood mononuclear cells with X-irradiated MT-2 cells as described previously (39).

Preparation of MAbs. Membrane fractions were prepared as described previously (15). Protein concentrations were determined by using the Bio-Rad protein assay kit (Japan Bio-Rad Laboratories, Tokyo, Japan). BALB/c mice were immunized intraperitoneally with membrane fractions (50 μg of protein per mouse) emulsified in complete Freund adjuvant once a week for 3 weeks. One week after the last injection, mice were put under anesthesia and membrane fractions (50 μg of protein) were injected into the spleens. Second intrasplenic immunizations were carried out after 1 week. Three days after the last immunization, spleen cells were fused with SP2 mouse myeloma cells by using polyethylene glycol 4000 and cultured in the presence of hypoxanthine-aminopterin-thymidine (7). Hybridomas were cloned by two successive limiting-dilution cultures (7). The immunoglobulin isotype of each MAb was determined by using an isotyping kit for mouse MAbs (Serotec, Oxford, United Kingdom). MAbs were purified by using the Affi-Gel Protein A MAPS-II kit (Japan Bio-Rad). Protein concentration was determined with the Bio-Rad protein assay kit (Japan Bio-Rad).

Syncytium formation assay. Syncytium formation between virus-positive cells and indicator MOLT-4-#8 cells (17) or HOS cells (23) was carried out essentially as described previously (18, 33). We used TOM-1 (18), TCL-Kan (34), and C91/PL (29a) for the assay of HTLV-1-induced cell fusion, and we used MOLT-4 persistently infected with HIV-1 (HTLV-IIIB) for the assay of HIV-1-induced cell fusion (17).

Flow cytometric analysis. Adherent cells were mechanically resuspended with a cell scraper after incubation at 37°C for 10 min in phosphate-buffered saline (PBS) containing 1 mM EDTA. Cells were washed in a blocking buffer (PBS containing 5% fetal bovine serum and 10 mM EDTA) and incubated at 4°C for 30 min in hybridoma culture supernatants. After washing, cells were incubated at 4°C for 30 min in the blocking buffer containing fluorescein-conjugated anti-mouse immunoglobulin G (IgG) (purchased from Kirkegaard & Perry, Gaithersburg, Md.). After washing, the cells were resuspended in blocking buffer containing propidium iodide, and fluorescence intensity was analyzed on a FACStar.

Immunoprecipitation and immunoblotting. Cell surface iodination was carried out by using lactoperoxidase (purchased from Sigma, St. Louis, Mo.) and carrier-free $Na^{125}I$ (purchased from Amersham, Buckinghamshire, United Kingdom). Metabolic labeling was carried out in methionine- and cysteine-free RPMI supplemented with 10% dialyzed fetal bovine serum and 200 μCi of [^{35}S]methionine and -cysteine mixture (purchased from American Radiolabeled Chemicals, St. Louis, Mo.) per ml for 15 min and was followed by a chase in RPMI supplemented with 10% fetal bovine serum for various lengths of time. In some experiments, tunicamycin or monensin (both purchased from Sigma) was included in the culture. Treatment with these drugs was started 2 h before the pulse-labeling and continued until the end of the culture. After washing, cells were lysed in lysis buffer (10 mM Tris-HCl [pH 8.0], 0.14 M NaCl, 3 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, 0.5% Nonidet P-40). Nuclei were removed by centrifugation, and the cell extracts were precleaned with protein G-Sepharose (purchased from Pharmacia). Then 100 μl of the extract was mixed with 100 μl of each hybridoma culture supernatant. After incubation at 4°C for 3 h, immune complexes were trapped by incubation with protein G-Sepharose (25-μl packed volume) at 4°C for 1 h with continuous rotation. The gels were extensively washed with lysis buffer. Bound materials were eluted with Laemmli's electrophoresis sample buffer, separated by 10 to 20% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and visualized by autoradiography or fluorography using Enlightning (NEN, Boston, Mass.).

Immunoblotting was carried out as follows. Cells were lysed in 50 μl of lysis buffer for 5 min at 4°C. After centrifugation at 1,200 rpm for 2 min, supernatants were mixed with 50 μl of Laemmli's electrophoresis sample buffer without 2-mercaptoethanol and boiled for 5 min. After SDS-PAGE (2×10^5 cell equivalents per lane), cell proteins were electrophoretically transferred to a membrane (Clear Blot Membrane-P, ATTO, Tokyo, Japan) and reacted with hybridoma supernatants (1:50 dilution). Bound MAbs were then reacted with horseradish peroxidase-conjugated anti-mouse IgG (heavy plus light chain) (Vector, Burlingame, Calif.) and visualized by chemiluminescence and fluorography using an ECL Western immunoblotting kit (Amersham).

Deglycosylation. *N*-Glycanase and *O*-glycanase were purchased from Genzyme (Boston, Mass.). Neuraminidase from *Arthrobactor ureafaciens* was purchased from Nacalai Tesque (Kyoto, Japan). Cells were surface labeled with ^{125}I, solubilized, and incubated with MAb C33. Immune complexes were trapped with protein G-Sepharose and extensively washed. Treatment with neuraminidase, with *O*-glycanase after neuraminidase, or with *N*-glycanase was carried out according to the protocol recommended by the manufacturer. Control and enzyme-treated proteins were analyzed by SDS-PAGE and autoradiography as described above.

RESULTS

Isolation of MAbs. A total of about 30,000 hybridoma supernatants were assayed for inhibitory activity to HTLV-1-mediated syncytium formation induced in coculture of a very sensitive indicator line, MOLT-4-#8 (17), with an HTLV-1-positive T cell line, TOM-1 (18) or C91/PL (29a). We obtained four MAbs, M38 (IgG1), M101 (IgG1), M104 (IgG1), and C33 (IgG2a), with such activities. They were derived from immunization with either HTLV-1-negative MOLT-4 (M series) or HTLV-1-positive C91/PL (C series). As shown in Fig. 1, the inhibitory activity of each MAb was apparently specific for HTLV-1, since none of these MAbs inhibited syncytium formation induced in coculture of the same indicator, MOLT-4-#8, with MOLT-4 persistently infected with HIV-1. As expected, H61 (IgG1), a MAb to human CD4 (data not shown), inhibited syncytium formation mediated by HIV-1 but not that mediated by HTLV-1 (3, 20). The maximum inhibitory effect of M104 and C33 was obtained at a concentration of 50 ng of each purified immunoglobulin per ml. We also tested the inhibitory effect of each MAb on syncytium formation induced in another indicator cell line, HOS (23), a human osteosarcoma line growing as a monolayer. Unexpectedly, none of these MAbs, including purified M104 and C33, even at a concentration of 50 μg/ml, either alone or in combination, inhibited syncytium formation induced in the coculture of HOS and C91/PL. The syncytium assay using HOS as indicator cells, however, seemed to be much less sensitive to the antibody-mediated

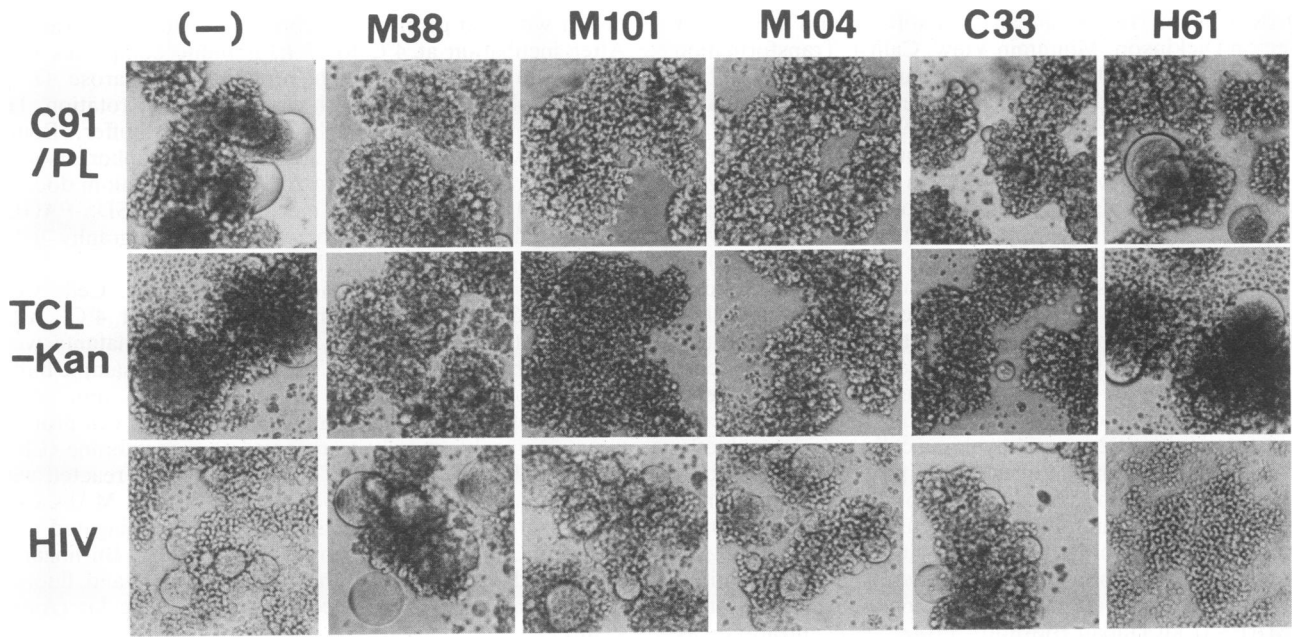

FIG. 1. Effects of MAbs M38, M101, M104, C33, and H61 on the syncytium formation induced by HTLV-1 and HIV-1. The syncytium formation assay was carried out by cocultivation of MOLT-4-#8 and C91/PL (HTLV-1 positive), TCL-Kan (HTLV-1 positive), or MOLT-4 (HIV-1 positive) for 24 h without or with each hybridoma supernatant (final concentration, 33%). For details, see Materials and Methods.

inhibition than that using MOLT-4-#8, since an adult T-cell leukemia serum containing antibodies to HTLV-1 envelope proteins completely inhibited syncytium formation at a dilution of 3,200-fold in the assay using MOLT-4-#8 but only at a dilution of 100-fold in the assay using HOS. These results are summarized in Table 1.

Reactivity of MAbs to various cell lines. We examined the cell reactivity of the four MAbs by indirect immunofluorescence staining and flow cytometric analysis (Table 2). Even though there were some differences in the level of staining, all of the MAbs reacted with various HTLV-1-negative human cell lines. These included not only T-cell lines but also B, myeloid, fibroblast, and epithelial cell lines. The reactivities of M101, M104, and C33 were restricted to human cells. M38 was further capable of staining feline and rabbit cells. All four MAbs also reacted with HTLV-1-positive human T-cell lines. M38 further stained an HTLV-1-positive feline cell line, C77 (13). Among these cell lines, only MOLT-4-#8 and HOS were readily induced to form numerous syncytia upon coculture with HTLV-1-positive T-cell lines in our hands (data not shown). These results, therefore, indicate that the antigens recognized by our MAbs are ubiquitous membrane molecules, do not quantitatively correlate with cellular inducibility for syncytium formation, and do not undergo down-regulation in HTLV-1-positive T-cell lines.

Reactivity of MAbs to mouse A9 clones having a single human chromosome. To determine chromosomal localizations of genes involved in the expression of the membrane antigens recognized by these four MAbs, we examined their reactivities to five murine A9 clones having different single human chromosomes (21). As shown in Table 3, all of the MAbs reacted with A9(3552)-2, which contained fragments from human chromosomes 11 (q23-pter) and X (qter-q26). Since A9(3884)-1, which was not stained by these MAbs, also contained the same X-chromosome fragment (qter-q26),

TABLE 1. Effects of MAbs and human sera on syncytium formation mediated by HTLV-1 and HIV-1

Antibody	Inhibition of syncytium formation[a] in coculture of:				
	TOM-1 (HTLV-1+) and MOLT-4-#8	TCL-Kan (HTLV-1+) and MOLT-4-#8	C91/PL (HTLV-1+) and MOLT-4-#8	MOLT-4 (HIV-1+) and MOLT-4-#8	C91/PL (HTLV-1+) and HOS
M38	+	++	++	−	−
M101	++	++	++	−	−
M104	++	+++	+++	−	−
C33	++	++	++	−	−
H61	−	−	−	+++	−
Adult T-cell leukemia serum	+++	+++	+++[b]	−	+[c]
Normal serum	−	−	−	−	−

[a] Arbitrarily indicated from − to +++ according to the following criteria: −, no inhibition; +, size of syncytium reduced; ++, number and size of syncytium reduced; +++, complete inhibition.
[b] Complete inhibition was achieved at a 3,200-fold dilution.
[c] Complete inhibition was achieved at a 100-fold dilution.

TABLE 2. Reactivity of MAbs to various cell lines

Cell line	Species	Cell type[a]	Relative staining intensity with MAb[b]:				
			M38	M101	M104	C33	H61
HTLV-1 negative							
MOLT-4	Human	T	+++	+++	+++	+++	+++
Jurkat	Human	T	+++	+++	+++	+++	++
CEM	Human	T	+++	+++	+++	+++	+++
HUT78	Human	T	++	+++	+++	+++	++
Raji	Human	B	+++	+++	+++	+++	ND[c]
Jijoye	Human	B	+++	++	+++	+++	ND
HL-60	Human	M	++	++	+++	+++	ND
U937	Human	M	+++	+++	+++	+++	+++
HeLa	Human	E	++	−	+	+	−
HEp-2	Human	E	++	++	++	++	−
HOS	Human	F	++	++	+++	+++	−
COS-7	Simian	F	−	−	−	−	−
Vero	Simian	F	−	−	−	−	−
MDCK	Canine	E	−	−	−	−	ND
8C	Feline	F	+++	−	−	−	ND
RK-13	Rabbit	E	++	−	−	−	−
NIH 3T3	Murine	F	−	−	−	−	−
HTLV-1 positive							
MT-1	Human	T	++	++	+++	+++	−
MT-2	Human	T	+++	+++	++	+++	+++
TOM-1	Human	T	+++	+++	+++	+++	ND
C91/PL	Human	T	+++	+++	+++	+++	+++
TCL-Kan	Human	T	+++	+++	+++	+++	+++
HUT102	Human	T	+++	+++	++	+++	ND
C77	Feline	E	+++	−	−	−	ND

[a] T, T cell; B, B cell; M, myelomonocytoid cell; E, epithelial; F, fibroblastoid. The level of reactivity was determined by indirect immunofluorescence staining and FACStar analysis.
[b] +++, strongly positive; ++, moderately positive; +, weakly positive; −, negative.
[c] ND, not done.

the responsible gene(s) must be localized on human chromosome 11 q23-pter.

Immunoprecipitation and immunoblotting by MAbs. To identify the membrane antigens recognized by these MAbs, we carried out immunoprecipitation experiments using cell extracts prepared from the surface-iodinated human T-cell lines Jurkat (HTLV-1 negative) and C91/PL (HTLV-1 positive). As shown in Fig. 2A, C33 immunoprecipitated surface molecules of 41 to 50 kDa from Jurkat (lane 3) and more heterogeneous molecules of 44 to 70 kDa from C91/PL (lane 7) under reducing conditions. M104 immunoprecipitated the same antigen as C33, though much more weakly (lanes 2 and 6). M38 (lanes 1 and 5) and M101 (data not shown) produced no detectable immunoprecipitation bands from either cell line. The sizes of the bands immunoprecipitated by C33 and M104 remained essentially unchanged under nonreducing conditions (data not shown). Figure 2B shows the results of similar experiments in which the immunoprecipitation with C33 was carried out for three other human T-cell lines, CEM (lanes 1 and 2), MT-2 (lanes 3 and 4), and TCL-Kan (lanes 5 and 6). Again, the antigen recognized by C33 was more heterogeneous in HTLV-1-positive MT-2 (lane 4, 43 to 73 kDa) and TCL-Kan (lane 6, 41 to 73 kDa) than in HTLV-1-negative CEM (lane 2, 42 to 54 kDa). We also carried out immunoblotting experiments to detect antigens in cellular extracts transblotted to membranes. C33 was capable of revealing the same antigen identified by immunoprecipitation experiments (data not shown). The reactivity of M104 was again much weaker if present at all, whereas the other two MAbs could not demonstrate any reacting antigens. It was also noted that cell extracts had to be electrophoresed under nonreducing conditions, in order to retain the reactivity of the antigen with C33, indicating the importance of a disulfide bond(s) for the epitope structure recognized by C33.

TABLE 3. Reactivity of MAbs to mouse A9 clones containing a single human chromosome (an X/autosome translocation)

Clone	Human chromosome	Reactivity[a]			
		M38	M101	M104	C33
A9(0439)-1	Xpter-Xq26::3p12-pter	−	−	−	−
A9(3552)-2	Xqter-Xq26::11q23-11pter	++	++	++	++
A9(2621)-4	Xqter-Xq22::12q24-12pter	−	−	−	−
A9(3884)-1	Xqter-Xq26::16q24-16pter	−	−	−	−
A9(7151)-3	Xqter-Xq11::17q11-17pter	−	−	−	−

[a] The reactivity of each MAb to A9 clones was determined by indirect immunofluorescence staining and FACStar analysis. See Table 1 for details.

It was of great interest to determine whether the presence of the HTLV-1 genome would induce the change in the size of the antigen recognized by C33 in the same cell background. However, our repeated trials to transmit the HTLV-1 genome into HTLV-1-negative human T-cell lines by cocultivation with X-ray-irradiated MT-2 cells were unsuccessful. Therefore, we freshly established human T-cell lines from peripheral blood mononuclear cells of healthy adult donors by cocultivation with X-ray-irradiated MT-2 cells. These cell lines, which were confirmed to be positive for CD4, interleukin-2 receptors, and HTLV-1 antigens (data not shown), were compared with fresh CD4-positive T cells prepared from the same donors. The immunoblotting

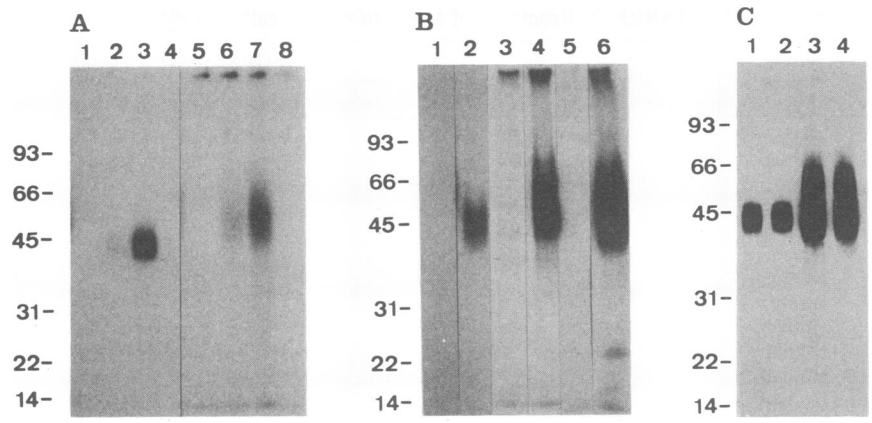

FIG. 2. Identification of the surface antigen(s) recognized by MAbs. (A) Immunoprecipitation from surface ^{125}I-labeled Jurkat (lanes 1 to 4) and C91/PL (lanes 5 to 8) with M38 (lanes 1 and 5), M104 (lanes 2 and 6), C33 (lanes 3 and 7), and a control MAb 8C3 (anti-human papillomavirus type 16 E7 protein [unpublished data]) (lanes 4 and 8). (B) Immunoprecipitation from surface ^{125}I-labeled CEM (lanes 1 and 2), MT-2 (lanes 3 and 4), and TCL-Kan (lanes 5 and 6) with a control MAb, 8C3 (lanes 1, 3, and 5), or C33 (lanes 2, 4, and 6). (C) Immunoblotting with C33 of cell extracts prepared from CD4-positive fresh T cells (lanes 1 and 2) or CD4-positive T-cell lines from the same donors established with HTLV-1 (lanes 3 and 4). The sizes of marker proteins are indicated in kilodaltons. For details, see Materials and Methods.

method was used instead of immunoprecipitation, since surface iodination or metabolic isotope labeling required a large number of fresh CD4-positive T cells. As shown in Fig. 2C, the antigen detected by C33 was 43 to 52 kDa in fresh CD4-positive T cells obtained from two donors and 41 to 66 kDa in HTLV-1-transformed CD4-positive T-cell lines derived from the same donors. These results showed that the size changes in the antigen recognized by C33 were also induced in normal CD4-positive T cells by transformation with HTLV-1.

Posttranslational modifications of the antigen by glycosylations. The dramatic differences in the molecular size of the surface antigen precipitated by C33 between HTLV-1-negative and -positive cells prompted us to analyze its intracellular processing by pulse-labeling and chasing experiments using HTLV-1-negative MOLT-4 and HTLV-1-positive C91/PL (Fig. 3). The antigen was first synthesized as a 35-kDa precursor during the 15-min labeling in both MOLT-4 and C91/PL (Fig. 3A, lanes 1 and 5, respectively). During the 5-h chase, the antigen was processed to 41 to 50 kDa in MOLT-4 (lanes 2 to 4) and to a more heterogeneous 44 to 70 kDa in C91/PL (lanes 6 to 8). In the presence of tunicamycin, an inhibitor of N-linked glycosylation (4), the antigen was synthesized as a 28-kDa protein core in both MOLT-4 and C91/PL (Fig. 3B, lanes 2 and 4, respectively). In the presence of monensin, which blocks intracellular transport into the Golgi apparatus (36), where further maturations of N-linked sugars as well as O-linked glycosylations take place, the processing from 35 kDa to 41 to 50 kDa in MOLT-4 and from 35 kDa to 44 to 70 kDa in C91/PL was inhibited (Fig. 3C, lanes 2 and 4, respectively).

Deglycosylation of the antigen. The apparent differences in the glycosylation pattern of the antigen recognized by C33 between HTLV-1-negative and -positive human T cells might arise from differences in the maturation of N glycosylation and/or additional O glycosylations taking place in the Golgi apparatus. To test these possibilities, the antigen was immunoprecipitated from extracts of surface-iodinated Jurkat (HTLV-1 negative) and C91/PL (HTLV-1 positive) and treated with various deglycosylation enzymes. As shown in Fig. 4, the size of the antigen was slightly reduced by treatment with neuraminidase in both cell lines (lanes 2 and 6), indicating the presence of terminal sialic acids. No further changes were seen by the subsequent treatment with *O*-glycanase (lanes 3 and 7). On the other hand, treatment with *N*-glycanase alone apparently removed oligosaccharide moieties completely from the antigen in both cell lines (lanes 4 and 8). These results indicated that the antigen had only N-linked oligosaccharide moieties with terminal sialic acids and that the differences in size observed between HTLV-1-negative and -positive T-cell lines were mostly due to differences in the maturation pattern of N glycosylation.

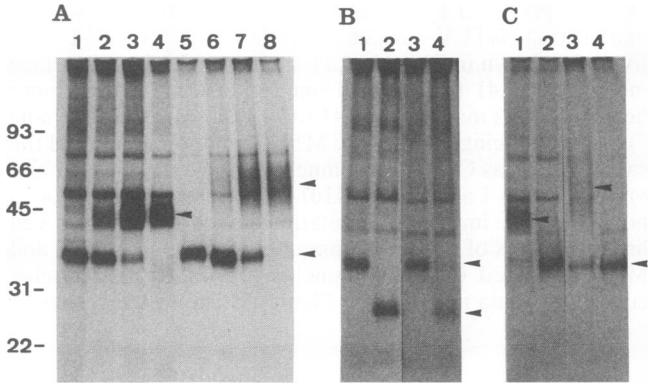

FIG. 3. Intracellular processing of C33 antigen in HTLV-1-negative MOLT-4 and HTLV-1-positive C91/PL. Immunoprecipitation was carried out with MAb C33. (A) MOLT-4 (lanes 1 to 4) and C91/PL (lanes 5 to 8) were metabolically labeled for 15 min and chased for 0 min (lanes 1 and 5), 30 min (lanes 2 and 6), 1 h (lanes 3 and 7), or 5 h (lanes 4 and 8). (B) MOLT-4 (lanes 1 and 2) and C91/PL (lanes 3 and 4) were metabolically labeled for 15 min without (lanes 1 and 3) or with (lanes 2 and 4) 10 μg of tunicamycin per ml. (C) MOLT-4 (lanes 1 and 2) and C91/PL (lanes 3 and 4) were pulse-labeled for 15 min and chased for 60 min without (lanes 1 and 3) or with (lanes 2 and 4) 10 μg of monensin per ml. The sizes of marker proteins are indicated in kilodaltons. For details, see Materials and Methods.

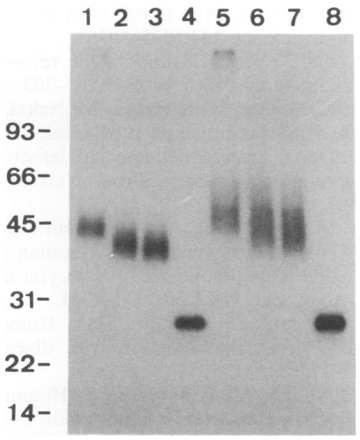

FIG. 4. Enzymatic deglycosylation of C33 antigen from HTLV-1-negative Jurkat and HTLV-1-positive C91/PL. Following immunoprecipitation from ^{125}I-labeled Jurkat (lanes 1 to 4) and C91/PL (lanes 5 to 8), C33 antigen was treated with no enzyme (lanes 1 and 5), neuraminidase (lanes 2 and 6), neuraminidase and *O*-glycanase (lanes 3 and 7), or *N*-glycanase (lanes 4 and 8). The sizes of marker proteins are indicated in kilodaltons. For details, see Materials and Methods.

DISCUSSION

The very low infectivity of cell-free HTLV-1 (1, 2, 26, 38) suggests that a close cell-to-cell interaction between virus-producing cells and target cells is important for transmission of the virus in vitro as well as in vivo (11, 25, 28, 39). The cell-to-cell interaction between HTLV-1-positive cells and certain target cells also induces syncytium formation (9, 13, 26). It is thought that these two phenomena are at least partly based on the same mechanism. Both transmission and syncytium formation are dependent on HTLV-1 *env* glycoproteins (19, 27, 38). In this study, we have isolated four MAbs which are capable of inhibiting HTLV-1-induced syncytium formation in a human T-cell line, MOLT-4-#8 (17) (Fig. 1 and Table 1). Their inhibitory activities were, however, not demonstrable when a human osteosarcoma cell line, HOS (23), was used as the source of syncytium indicator cells (Table 1). Even though we have not yet fully proven the possibility, all of these MAbs may recognize the same membrane antigen, since they all reacted with the same mouse A9 clone containing a part of human chromosome 11 (21) (Table 3), and at least two of them (M104 and C33) immunoprecipitated a membrane antigen with the same molecular size (Fig. 2). There were, however, some differences in their fusion-inhibitory activity, depending on which HTLV-1-positive T-cell line was used in the assay (Table 1), and in their immunostaining intensities with various cell lines (Table 2). M38 was even capable of staining some non-human cell lines. The binding of C33 was partially blocked by M104 but not by two other MAbs (data not shown). Therefore, even if they react with the same membrane antigen, their recognizing epitopes must be different.

Even though syncytium formation between retrovirus-infected cells and target cells is widely used to monitor virus-receptor interactions, it is in fact a highly complex phenomenon. Not only the interactions between *env* proteins and specific cell surface receptors but also those of certain cell adhesion molecules may be required for a strong cell-to-cell interaction leading to cell fusion. The lipid composition of the target cell membrane may also be important (31a). The activation state of target cells may also affect syncytium formation (1a, 25a). In the case of HIV-1, syncytium formation is now known to depend not only on the HIV-1 receptor CD4 (3, 20) (Fig. 1 and Table 1) but also on certain cell adhesion molecules such as LFA-1 and some other leukocyte cell adhesion molecules (10, 36a). It was shown that the activation of protein kinase C was inhibitory to HIV-1-induced syncytium formation in MOLT-4 cells (1a) but was required for fresh CD4-positive T cells to become fusogenic (25a). Our MAbs, which are inhibitory to HTLV-1-induced syncytium formation in MOLT-4-#8, might, therefore, recognize either the HTLV-1 receptor itself, which is now known to be widely distributed among different cell types and host species (27, 38), or a membrane molecule(s) specifically involved in the process of HTLV-1-induced syncytium formation in human T cells, either directly, such as by promoting cell adhesion, or indirectly, such as by affecting cellular competence for fusion by modulating the cellular activation state.

The surface antigen identified by C33 and M104 (henceforth called C33 antigen) is not apparently down-regulated in HTLV-1-positive cell lines which produce HTLV-1 *env* glycoproteins (Table 2). There were, however, notable differences in the size of C33 antigen between HTLV-1-positive and -negative human T cells. C33 antigen was more heterogeneous in the former than in the latter (Fig. 2 and 3). C33 antigen was first synthesized as a 35-kDa glycoprotein which was composed of a 28-kDa core protein and tunicamycin-sensitive N-linked oligosaccharides (4). Further modifications which were sensitive to monensin and therefore apparently took place in the Golgi apparatus (36) converted the 35-kDa form into an approximately 40- to 55-kDa form in HTLV-1-negative T cells and into an approximately 40- to 75-kDa form in HTLV-1-positive T cells (Fig. 3). Since the sugar moieties of C33 antigen were completely removed by *N*-glycanase (Fig. 4), the differential modifications observed in HTLV-1-negative and -positive T cells must be mostly due to differences in the pattern of maturation of N-linked oligosaccharides in the Golgi apparatus. The structural difference in N-linked glycosylations and the underlying mechanism for such alterations in HTLV-1-positive cells remain to be determined, but the latter may be related to the malignancy-associated increase in β1-6-branched N-linked complex-type oligosaccharides which are known to be induced following transformation with chemical carcinogens, oncogenic viruses, or activated oncogenes (30). It is also possible that altered forms of N-linked oligosaccharides in C33 antigen are selected for during the process of transformation and outgrowth of HTLV-1-infected T cells in order to reduce self-catalyzed syncytium formation without down-regulation of C33 antigen (2). Experiments to test these possibilities are in progress.

From the study of infectivity of pseudotype vesicular stomatitis virus bearing HTLV-1 envelope glycoproteins [VSV (HTLV-1)] to human-mouse somatic cell hybrids, Sommerfelt et al. showed that the susceptibility was determined by human chromosome 17 (cen-qter) (33a). However, it is not clear whether this result defines the gene encoding the HTLV-1 receptor protein itself or a modifying enzyme such as a glycosyltransferase required for presentation of the receptor in its biologically functional form. In the present study, we examined the reactivity of our fusion-inhibitory MAbs to five mouse A9 clones having a single human chromosome (21); we found that all four MAbs stained an A9 clone with human chromosome 11 (q23-pter) but that none of them reacted with an A9 clone with human chromosome 17

(q11-pter) (Table 3). We confirmed the presence of human chromosome 11 in A9(3552)-2 and the presence of human chromosome 17 in A9(7151)-3 by Southern blotting hybridizations using human Ha-*ras* and p53 genes as probes for human chromosomes 11 and 17, respectively (5, 16) (data not shown). At least one of the MAbs (C33) was shown to react with the nonglycosylated form of the antigen which was synthesized in the presence of tunicamycin (Fig. 3). Therefore, it is likely that the gene on human chromosome 11 encodes the protein moiety of C33 antigen and not a processing enzyme. The finding that our MAbs could not inhibit HTLV-1-induced syncytium formation in another indicator line, HOS (Table 1), however, may argue against C33 antigen as the cellular receptor for HTLV-1. It remains to be seen whether our MAbs are capable of inhibiting infection of VSV(HTLV-1) pseudovirus or HTLV-1-induced transformation of fresh human T cells upon coculture. Isolation of the gene encoding C33 antigen and its expression in appropriate host cells may also aid our insight into the mechanism of syncytium formation and the mode of transmission of HTLV-1.

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